Flow of concentrated red blood cell suspensions at micro-bifurcations: an *in vitro* experimental study

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1. Introduction

Blood is a concentrated suspension ($\sim$45% volume fraction) of deformable red blood cells (RBCs) flowing in a Newtonian fluid called plasma. In the microcirculation, vessel diameters are typically $<100\,\mu$m. Thus, the characteristic size of the RBCs ($\approx 10\,\mu$m) is not negligible compared with the vessel size. For these reasons, the dynamical effects due to the presence of RBCs are important in microvascular flows which induce nonlinear complex rheological behaviours. In particular, at diverging microvascular bifurcations, RBCs and plasma may be distributed non-proportionally between the two daughter vessels: one gets a higher RBC volume fraction (haematocrit) than the feeding vessel, whereas the other gets a lower RBC volume fraction. This effect, known as the phase separation effect, causes a tremendous heterogeneity of the haematocrit among vessels in microvascular networks. It induces a coupling between microvascular architecture, blood flow dynamics and exchanges of oxygen and nutrients between blood and tissues, which take place at microvascular level.

In order to properly describe and understand such a coupling, a quantitative description of the phase-separation effect is needed.

The aim of this work was to investigate the phase-separation effect *in vitro* for channels $<20\,\mu$m and for intermediate haematocrits, that is physiological conditions under which the effect is significant. For this purpose, we use RBC suspensions, microfluidic devices modelling microvascular bifurcations and new metrologies allowing the simultaneous quantification of both blood and RBC flow rates in the three branches of the bifurcation.

First, these metrologies were validated by checking that their results are consistent with mass conservation of both blood and RBCs at microbifurcations. Second, the phase separation effect was studied, and the result compared with previous experimental and numerical results (Pries et al. 1989; Doyeux et al. 2011).

2. Methods

Microchannels are made of a unique multidepths microbifurcation composed of one inlet and two outlet segments of square cross sections ($10\,\mu\text{m} \times 10\,\mu$m or $20\,\mu\text{m} \times 20\,\mu$m in poly-(dimethylsiloxane), see Figure 1).

Blood samples of controlled haematocrit are prepared. The flow is generated in the microsystem using a precision pressure control method. More detail on the experimental protocol can be found in Roman et al. (2012).

RBCs maximal velocity profiles are measured by the optimised *dual-slit* technique (Roman et al. 2012), a temporal correlation method. Two different methods were used to measure the haematocrit: a counting method is used when the suspension is diluted (haematocrit $<8\%$); a photometric method is used for higher concentrations. RBCs and suspending fluid flow rates are then directly deduced from these velocity and haematocrit measurements: the tridimensional velocity profile of RBCs is deduced from the maximal velocity profile of RBCs, integrated over the channel cross section and multiplied by the measured haematocrit to obtain the RBC flow rate (Q_{GR}). The suspending fluid flow rate is determined assuming Newtonian behaviour, a maximal suspending fluid velocity equal to the measured maximal RBC velocity and no slip at walls (Roman 2012).

Finally, the position of RBC flow separating line between both daughter branches is determined using spatial correlation algorithms (e.g. PIV (particle image velocimetry): ImageJ plug-in).

3. Results and discussion

RBC flow at microbifurcation was explored in a large range of experimental conditions: measured velocities ranged from 7 to 1500 μm/s, the haematocrits were between 0.1% and 22% and the blood flow rates were from 7×10^3 to $8.5 \times 10^5\,\mu\text{m}^3/\text{s}$.

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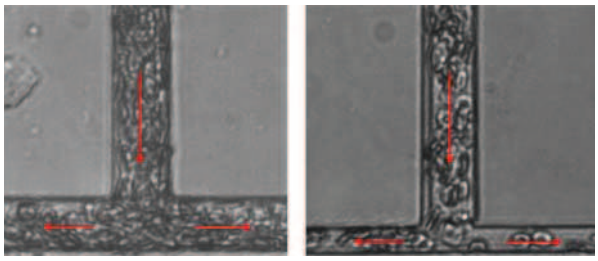

Figure 1. RBCs flowing in microbifurcations, composed of three 20 μm × 20 μm channels (left) and of one 20 μm × 20 μm inlet and two 10 μm × 10 μm outlets.

3.1 RBC velocity profiles

Our results show the existence of two flow regimes which depend on the size of the microchannel. When the size of the channel is close to the size of a single RBC (10 μm), all RBCs move at the same velocity, even at large concentrations by which they are not organised in single file anymore. On the contrary, when the channel is larger, velocity gradients appear: RBCs are slower near the channel's wall than in the centre, with a non-zero slip velocity at the walls.

3.2 Mass conservation

At first, the principle of mass conservation was verified between the three branches of the bifurcation by comparing the values of the measured flow rates into and from the bifurcation. *In vivo* data have been compiled by Cokelet et al. (1998) to quantify the experimental error by studying the observed deviations from the principle of mass conservation. These *in vivo* data are compared to our *in vitro* data given in Figure 2. The results show that the methodologies developed to measure blood and RBC flows in this work are good and very accurate compared with previous work.

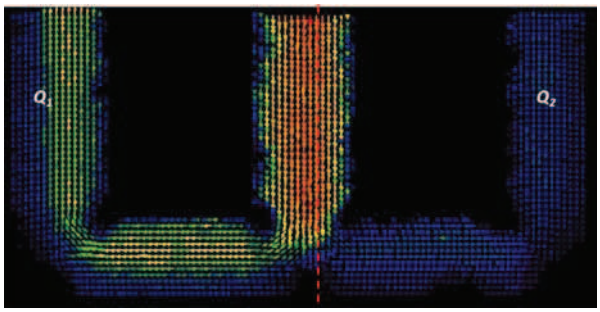

Figure 3. PIV vectors showing, qualitatively, RBC flow direction at a bifurcation composed of three 20 μm × 20 μm channels, $Q_1 > Q_2$, red-dotted line: RBC separating line.

3.3 Phase separation effect

The results suggest the existence of two regimes in the description of the phase separation effect. When the bifurcation consists of three 20 μm × 20 μm channels, the influence of the haematocrit on the phase separation effect is pronounced. In this case, the effect indeed disappears at large haematocrit (> 8%). When the bifurcation is composed of an inlet channel of size 20 μm × 20 μm and two outlets of size 10 μm × 10 μm, there is no influence of the haematocrit on the phase separation effect. In addition, in this second case, the phase separation effect is greater (about 1020%) than what has been previously described *in vivo* (Pries et al. 1989) or numerically (Doyeux et al. 2011), whereas in the first case, the orders of magnitude are the same as these previous data. These differences could be related to the different flow regimes of RBCs observed in 20 μm × 20 μm and 10 μm × 10 μm channels.

3.4 Position of RBC separating line

With PIV measurements, the position of the RBC flow separating line can be evaluated (Figure 3). This position

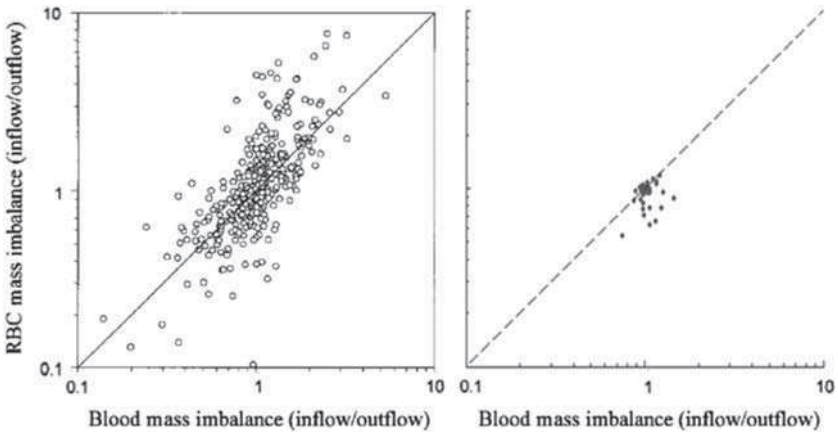

Figure 2. Quantification of deviations to mass conservation: left, Cokelet compilation from *in vivo* data; right, present work.

was compared with the position predicted for isolated and circular particles at a bifurcation, in the literature (Doyeux et al. 2011). The influence of haematocrit on the position of the dividing line may also be assessed.

4. Conclusions

The experimental device and the methodologies developed provide accurate quantitative data on the flow of RBCs at microbifurcations. Relevant data on the flow of RBCs in microchannels (Roman 2012) and preliminary results on the phase separation effect were obtained for channel sizes and haematocrit ranges never studied in controlled flow conditions. These encouraging findings will be further developed, especially through the study of the position of RBC separating line.

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